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Article

Elevated Atmospheric CO_2 Affects Ectomycorrhizal Species Abundance and Increases Sporocarp Production under Field Conditions

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Abstract: Anthropogenic activities during the last century have increased levels of atmospheric CO_2. Forest net primary productivity increases in response to elevated CO_2, altering the quantity and quality of carbon supplied to the rhizosphere. Ectomycorrhizal fungi form obligate symbiotic associations with the fine roots of trees that mediate improved scavenging for nutrients in exchange for a carbohydrate supply. Understanding how the community structure of ectomycorrhizal fungi is altered by climate change is important to further our understanding of ecosystem function. *Betula pendula* and *Fagus sylvatica* were grown in an elevated CO_2 atmosphere delivered using free air carbon dioxide enrichment (FACE) under field conditions in the U.K., and *Picea abies* was grown under elevated CO_2 in glass domes in the Czech Republic. We used morphotyping and sequencing of the internal transcribed spacer region of the fungal ribosomal operon to study ectomycorrhizal

community structure. Under FACE, un-colonised roots tips increased in abundance for *Fagus sylvatica*, and during 2006, sporocarp biomass of *Peziza badia* significantly increased. In domes, ectomycorrhizal community composition shifted from short-distance and smooth medium-distance to contact exploration types. Supply and competition for carbon belowground can influence ectomycorrhizal community structure with the potential to alter ecosystem function.

Keywords: FACE; community structure; root tips; forest; hyphae; rhizomorph; morphotype; internal transcribed spacer (ITS); sequence

1. Introduction

The fine roots tips of trees in boreal forest, such as spruce, fir and pine [1] and in temperate forests, such as beech and hornbeam [2], are almost completely colonised by ectomycorrhizal fungi. The fine roots of most trees are colonised by a large number of ectomycorrhizal fungal species [2–4]. Determination of the community structure of ectomycorrhizas is carried out by analysis of fine root tips [2,5]. Identification of the fungal species forming the ectomycorrhizas normally involves morphotyping [6] and a subsequent molecular identification [7,8]. Such investigations have shown that ectomycorrhizal communities in forest can be highly diverse, but that these communities tend to be dominated by a few species and have a high number of rare species [2–4]. The ectomycorrhizal community structure is influenced by tree species [2] and also by environmental factors, such as nutrients [9]. The high diversity of ectomycorrhizal fungi is thought to be important in maintaining ecosystem function [10–12]; however, advances in the understanding of ectomycorrhiza function [13,14] have shown a wide range of potential functional groups. Functional group diversity and changes in belowground community composition have been suggested to be more important for ecosystem functioning than species diversity alone [15].

Species of ectomycorrhizal fungi differ in the extent of their extramatrical mycelium, which allows the ectomycorrhizas to be divided into different exploration types [16]. Based on the amounts of emanating hyphae and the presence and differentiation of rhizomorphs, Agerer [16] defined five main exploration types, ranging from contact exploration types, with smooth mycorrhizal tips having only a few short emanating hyphae, via short and medium exploration types to the long-distance exploration type with highly differentiated rhizomorphs [17]. In many genera, all known species produce only one exploration type, e.g., species in most of the investigated genera of the Boletales belong to the long-distance exploration type that has hydrophobic rhizomorphs, while in other genera, e.g., *Russula* and *Lactarius*, the exploration types vary between different species and can form contact, short distance and medium distance types[14]. An ectomycorrhizal community has a range of exploration types [18], suggesting a degree of separation into functional traits [19].

Anthropogenic activities since the industrial revolution have increased atmospheric CO_2 concentrations [20]. Increased atmospheric CO_2 concentrations promote forest net primary production (NPP) [21]. As ectomycorrhizas are dependent on a C supply from the host tree, they are potentially affected by changes in the rates of photosynthesis and NPP. In agreement with this idea, both the extent

of ectomycorrhizal colonisation and the growth of the extramatrical mycelium [22] have been shown to be affected by exposure of the host to elevated CO_2. The extent of ectomycorrhizal colonisation has been used as a measure of the growth of ectomycorrhizal fungi relative to the growth of the host tree fine roots and has been shown under elevated CO_2 to both increase [23,24], but also to decrease [9]. In investigations under controlled conditions, elevated CO_2 has been shown to increase the growth of the extramatrical mycelium [25,26]. This response, however, is dependent on the tree [25] and fungal species [26]. In the work of Fransson *et al.* [26], fungal biomass production increased significantly with elevated CO_2 for two species (*Hebeloma velutipes* Bruchet and *Amanita muscaria* (L.) Lam.), whereas three species (*Piloderma fallax* (Lib.) Stalpers, *Paxillus involutus* (Batsch) Fr. and *Rhizopogon roseolus* (Corda) Th. Fr.) showed a similar, but non-significant trend. In contrast, *Laccaria bicolor* (Maire) P.D. Orton and *Piloderma byssinum* (P. Karst.) Jülich produced less biomass in elevated CO_2 compared to ambient CO_2.

Of the investigations of the effect of elevated CO_2 on fungal community structure of ectomycorrhizas, many have been carried out under controlled conditions. Some of these experiments have shown little effects of elevated CO_2 in ectomycorrhizal community structure. Rygiewicz *et al.* [27] planted two-year-old *Pseudotsuga menziesii* (Mirb.) Franco seedlings in chambers, and after four years of elevated CO_2 exposure found small overall effects on the ectomycorrhizal morphotype community structure. In a pot experiment, Markkola *et al.* [28] were unable to detect any changes in ectomycorrhizal community structure on roots of *Pinus sylvestris* L. as a result of a doubling of atmospheric CO_2 concentration. However, the roots of the *P. sylvestris* seedlings were only colonised by three different ectomycorrhizal morphotypes, and one single morphotype represented *ca.* 80% of the root tips. However, some studies have shown changes in the ectomycorrhizal community structure under elevated CO_2. Godbold *et al.* [25] showed in *Betula papyrifera* Marshall and *Pinus strobus* L. a change in the ectomycorrhizal community structure towards a greater frequency of ectomycorrhizas with emanating hyphae and rhizomorphs under elevated CO_2. Godbold and Berntson [29] suggested that higher supply of C to roots under elevated CO_2 promoted the occurrence of ectomycorrhizal morphotypes with a higher production of hyphae and rhizomorphs. The idea that some ectomycorrhizal fungi can preferentially benefit from a high C supply to the roots was supported by the observation that *Suillus bovinus* (L.) Roussel and *Laccaria bicolor* respond differently in the way that they partition assimilates between fungal biomass and respiration under elevated CO_2 [30]. However, these investigations under controlled conditions have been carried out on young tree seedling or saplings, which often are colonised by a restricted number of ectomycorrhizal species.

In field-based experiments with a potentially more diverse ectomycorrhizal community, community structure was altered in a 37 year-old *Picea abies* (L.) H. Karst. stand exposed to a doubling of atmospheric CO_2 concentration in whole tree chambers [9]. This was manifested mainly as a change in the abundance of a few common species. Rey and Jarvis [31] found indications that the mycorrhizal species composition in *Betula pendula* Roth shifted towards later successional stages after CO_2 treatment.

The above studies indicate that the response to elevated levels of CO_2 is tree and fungal species specific. The aims of the work presented here were to investigate whether in three different tree species, the previously observed response of the ectomycorrhizal community to elevated CO_2 levels occurs in both a field experiment and a field-based chamber experiment. In the work presented, CO_2 has been increased using free air carbon dioxide enrichment in field conditions or the CO_2 concentration has been

elevated in glass domes with adjustable windows surrounded by natural forest in a mountainous terrain. We hypothesized that elevated CO_2 would alter the ectomycorrhizal community structure towards species of a later seral stage with a greater C demand putatively via an increase in C availability to the roots.

2. Material and Methods

The investigation was carried out at two facilities manipulating atmospheric CO_2 concentrations, one using CO_2 enrichment in glass domes (Bílý Kříž) and the other using a free air carbon dioxide enrichment system (BangorFACE).

2.1. The Bílý Kříž Site

The Bílý Kříž experimental research site is situated in the Beskydy Mountains in the Czech Republic, (49°30′ N 18°32′ E, 908 m above sea level (asl)). The area was previously covered by managed *Picea abies* forest. The site has a continental subarctic/boreal climate with an annual mean air temperature of 6.7 °C, an annual mean relative air humidity of 80% and an average annual precipitation of 1374 mm for 2000–2009. The geological bedrock is Mesozoic Godula sandstone (flysch type), and the dominant soil type is a Ferrods. Two glass domes with adjustable windows (10 × 10 × 7 m) were established in 1995. One was maintained at ambient CO_2 concentration (365 to 377 ppm), whilst in the other dome, the concentration of atmospheric CO_2 was enriched to 700 ppm. An area without a dome was used to estimate the effects of the dome alone. The target concentration of 700 ppm was maintained within the range of 600–800 ppm for 72% of the time through the vegetation period. Before the first planting of the experiment, the disturbed top of the soil was supplemented and intermixed with a 10 cm-thick layer of peat to give a similar pH and organic matter content to that of the surrounding forest. The first plantings (*Picea abies* L. Karst.) were harvested in 2006. In 2007, a mixed culture of *Picea abies* L. Karst. and *Fagus sylvatica* L. was established using 3-year-old saplings planted at a density of 10,370 trees ha^{-1}. The experiment was finished in 2013, by which time, 9-year-old *Picea abies* trees had reached the height of 3.40 m in domes and 2.55 m in the unenclosed control area. Based on measurement in 1998 and 1999, there was no significant difference in air temperature between the domes and the unenclosed area over the whole year [32]. However, during the winter period, air temperature within the domes was significantly increased by between 0 and 4 °C for 60% of the time. Inside the domes, the length of time the soil was covered by snow was only 70% of that of the unenclosed areas. In summer, air relative humidity was significantly lower inside the domes than outside (−9.6% ± 5.3%). Soil moisture was significantly higher inside than outside the domes due to the artificial irrigation. Measurements, which were carried out during mixed culture experiment, were in accordance with the previous observation. There was slightly lower temperature (the average difference in monthly air maximum temperature was 1.5 and 2 °C, respectively) and slightly lower soil humidity (average difference 3% and 5%) of the non-enclosed control compared to the glass domes. Except for a lower amount of available K in the elevated CO_2 treatment, other soil parameters, such as pH, N and available nutrients, were not affected by enclosure or CO_2 treatment [33].

2.2. The BangorFACE Facility

The BangorFACE experimental site was established in March, 2004, on two former agricultural fields with a total area of 2.36 ha at Bangor University's research centre (53°14′ N, 4°01′ W) in North Wales, U.K. Both fields were originally pastures; one field was used for small-scale forestry experiments for the last 20 years; the other field was ploughed and planted with oil seed rape in 2003. Climate at the site is classified as hyperoceanic, with a mean annual temperature in 2005 through 2008 of 11.5 °C and an annual rainfall of 1034 mm. The soil is a fine loamy brown earth over gravel (Rheidol series) and classified as Fluventic Dystrochrept [34]. Soil texture is 63% sand, 28% silt and 9% clay. The topography consists of a shallow slope of approximately 1–2° on a deltaic fan. The site aspect is northwesterly, with an altitude of 13 to 18 m a.s.l. The depth of the water table ranges between 1 and 6 m.

At the BangorFACE site, eight octagonal plots, four ambient and four CO_2 enriched, were established, creating a 2 × 4 factorial block design across the two fields. Three tree species (*Alnus glutinosa* L. Gaertn., *Betula pendula* and *Fagus sylvatica*) were selected due to their contrasting shade tolerance, successional chronology and to represent a range of taxonomic, physiological and ecological types. Each plot was divided into seven planting compartments and planted in a pattern creating areas of one, two and three species mixtures [35]. The present study makes use of observations originating from the single species subplots of *B. pendula* and *F. sylvatica*. The site was planted with 60-cm saplings of each species. Within each treatment, the planting pattern was rotated by 90° between the four plots to avoid potential artefacts introduced by microclimate, soil and uneven growth rates of the different species. Each plot was surrounded by a 10-m border of *B. pendula*, *A. glutinosa* and *F. sylvatica* planted at the same density. The remaining field was planted at a 1-m hexagonal spacing with a mixture of birch (*B. pendula*), alder (*A. glutinosa*), beech (*F. sylvatica*), ash (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanus* L.), chestnut (*Castanea sativa* Mill.) and oak (*Quercus robur* L.). To protect the saplings, the entire plantation was fenced.

Carbon dioxide enrichment was carried out using high velocity pure CO_2 injection, with a target concentration in the FACE plots of ambient plus 200 ppm [34]. The elevated CO_2 concentrations, measured at 1-minute intervals, were within a 30% deviation from the pre-set target concentration of 580 ppm CO_2 for 75%–79% of the time during the photosynthetically active part of 2005–2008 [34]. Vertical profiles of CO_2 concentration measured at 50-cm intervals through the canopy showed a maximum difference of 7%.

2.3. Root Sampling and Identification of Ectomycorrhizas

2.3.1. Bílý Kříž

A total of 71 samples of *Picea abies* roots (23–25 samples per treatment) were collected in 2012–2013 (sampling dates: September 2012, May 2013, August 2013). A root sample consisted of 1–3 root fragments of total lengths of *ca.* 10 cm. *P. abies* roots were distinguished from *Fagus sylvatica* roots by morphology and anatomy, or in the case of the final sampling date, the roots were traced to a specific tree. At each sampling date, the samples were collected so that the entire plot area was evenly covered. Root samples were kept at 4 °C and were processed within three days of harvesting. All root fragments were carefully washed with tap water and then cleaned using tweezers to remove any residual

soil particles. They were observed under a stereomicroscope (SZX 12, Olympus, Tokyo, Japan) and documented (Camedia C5050, Olympus, Tokyo, Japan). All ectomycorrhizal tips were preliminary assigned to morphotypes according to [17]. Approximately three root tips per morphotype and treatment were selected for molecular identification and preserved at −20 °C. As the roots grown under elevated CO_2 had more clustered root tips, the number of root tips was higher in the elevated CO_2 treatment (2625 root tips) compared to root samples taken in the ambient dome (1892 root tips). A total of 2341 root tips were analysed from the unenclosed plot.

DNA was extracted using the Plant DNeasy Extraction kit (Qiagen GmbH, Hilden, Germany). PCR reactions were set up using standard protocols. DNA fragments spanning rDNA ITS were amplified using the primers ITS1F and ITS4, and low-quality products were reamplified using ITS1 and ITS4 [36]. Double products were amplified from genomic DNA again using specific primers for basidiomycetes (ITS1F, ITS4Basidio; [7,37]) and ascomycetes (ITS5, ITS4Asco; [7,37]) in order to separate products. PCR condition used an initial cycle of 150 s at 94 °C, followed by 37 cycles of 30 s at 94 °C, 40 s at 50 °C, 30 s at 72 °C and a 270-s final extension at 72 °C. The annealing temperature for primer ITS5 and ITS4Asco was 55 °C. Sequencing was done by Macrogen Inc., Seoul, Korea, utilizing an ABI 3730 XL automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence chromatograms were analysed using FinchTV program (Geospiza, Inc., Seattle, WA, USA) and aligned in ClustalX [38] with sequences of the best BLAST matches and related sequences from the public databases, Unite [39] and GenBank [40]. The sequences were deposited in GenBank with Accession No. KJ195334- KJ195446. A value of 98% ITS region sequence identity was used as a molecular species criterion. The identified fungal symbionts were used to further refine the preliminary morphotyping, and subsequently, further samples of poorly-delimited morphotypes were analysed and identified. As it was not possible to extract DNA from old, shrivelled ectomycorrhizas of sufficient quality for sequencing, these were classified as unidentified morphotypes. Identified morphotypes were assigned to the exploration type according to Agerer [41], Agerer and Rambold [17] and our own observations.

2.3.2. BangorFACE

Soil samples were taken in July, 2006, using an 8 cm in diameter corer to depths of 0–10 cm and 10–20 cm. Two replicate cores were taken in each of the four single-species subplots of *Betula pendula* and *Fagus sylvatica* for each treatment. The cores were kept moist and were stored at 4 °C until they were processed. To remove the roots from the cores, the soil cores were completely immersed in water for several minutes, and the roots were then carefully removed and gently agitated in water to remove the soil. Fine root tips were sampled from the larger root samples and placed under water in a Petri dish. The roots were scanned using WinRhizo (Version 2005c, Regent Instruments Inc., Quebec, Canada) to determine root length and the number of root tips. One hundred to 150 root tips were analysed from each replicate plot, giving a total of *ca.* 500 root tips per species per treatment.

Identification of ectomycorrhizal morphotypes was carried out under a dissecting microscope using an online identification key DEEMY [17], which is based on shape and dimensions, surface features and details of rhizomorphs and emanating hyphae. The morphotypes were documented using a Canon digital camera fitted to the microscope.

2.4. Collection of Sporocarps

In 2006 at BangorFACE, sporocarps were collected every 7–10 days from 1 September to 1 November. The sporocarps were identified to species [42], gently rinsed in water, dried at 70 °C and the dry weight determined.

2.5. Statistical Analysis

For the Bílý Kříž data, the structure of the communities was analysed using canonical correspondence analysis (CCA) and redundancy analysis (RDA) in Canoco 5 [43]. In CCA, the numbers of individual morphotypes were used as response data, site as an explanatory variable and date of collection as a covariable. The response data were square-root transformed, and down-weighting of rare species was applied. The structure of the communities based on exploration types was analysed by redundancy analysis (RDA), with numbers of root tips of exploration types (square-root transformed) used as response data and site and date of collection as an explanatory variable and covariable, respectively. The selection of the unimodal (CCA) or linear (RDA) multivariate method was made based on the length of the gradient from a previous detrended correspondence analysis. The effect of the site on the presence of morphotypes or exploration types was tested by the Monte Carlo permutation test.

While the number of analysed root tips varied between treatments, EstimateS [44] was used to compute a rarefaction curve ([45], Equation 17). Unidentified root tips were excluded from the dataset.

For the BangorFACE data, differences in the occurrence of morphotypes in each treatment were identified by one-way ANOVA with SPSS 14.0 (SPSS Inc. Chicago, IL, USA). The equality of variance was assessed using Levene's test, and normality was determined using the Shapiro-Wilk test following a log10 transformation. The total number of root tips was composed of both identified and unidentified ectomycorrhizas, non-mycorrhizal root tips and non-identifiable sub-vital or necrotic root tips.

3. Results

At the Bílý Kříž site, from the ectomycorrhizal root tips of *Picea abies*, 110 ITS rDNA fragments of fungi were successfully sequenced (Supplementary Materials, Table S1). Of these, ten sequences matched root endophytes (*Cadophora finlandica* (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew, *Phialophora fortinii* C.J.K. Wang & H.E. Wilcox) were excluded from further analysis. A total of 18 ectomycorrhizal fungal species, with 11–14 ectomycorrhizal species per treatment, was determined. An average of 2.7 ectomycorrhizal species was found per root sample, with a range of 1–5 species. According to rarefaction curves (sample-based approach; Supplementary Materials, Figure S1) after 1500 root tips had been analysed, approximately 11 species in ambient domes and in the unenclosed plot and 12 species in elevated CO_2 glass domes should have been detected. However, it is possible that some very rare species were still not detected.

The percentage colonization of different mycorrhizal species in each treatment is shown in Figure 1. Of the dominant species, *Amphinema byssoides* (Pers.) J. Erikss., *Piloderma* sp. 1 and *Trichophaea hybrida* (Sowerby) T. Schumach. were common in both the ambient and elevated CO_2 domes. In contrast, *Trichophaea* sp. 1 was common under elevated CO_2, but not under ambient conditions, whereas *Hydnotrya cerebriformis* (Tul. & C. Tul.) Harkn. was common in ambient conditions, but not

elevated CO_2. The unenclosed control was dominated by four species, *Amphinema byssoides*, *Piloderma* sp. 1, *Trichophaea hybrida* and *Tylospora asterophora* (Bonord.) Donk, but the species *Hydnotrya cerebriformis* and *Trichophaea* sp. 1 were not present. A higher proportion of species from the Thelephorales and the Atheliales and a smaller proportion from the Agaricales were found. Three additional species were found, *Thelephora terrestris* Ehrh., *Pseudotomentella mucidula* (P. Karst.) Svrcek and *Tomentella ellisii* (Sacc.) Jülich & Stalpers, which did not occur in the ambient or elevated CO_2 domes.

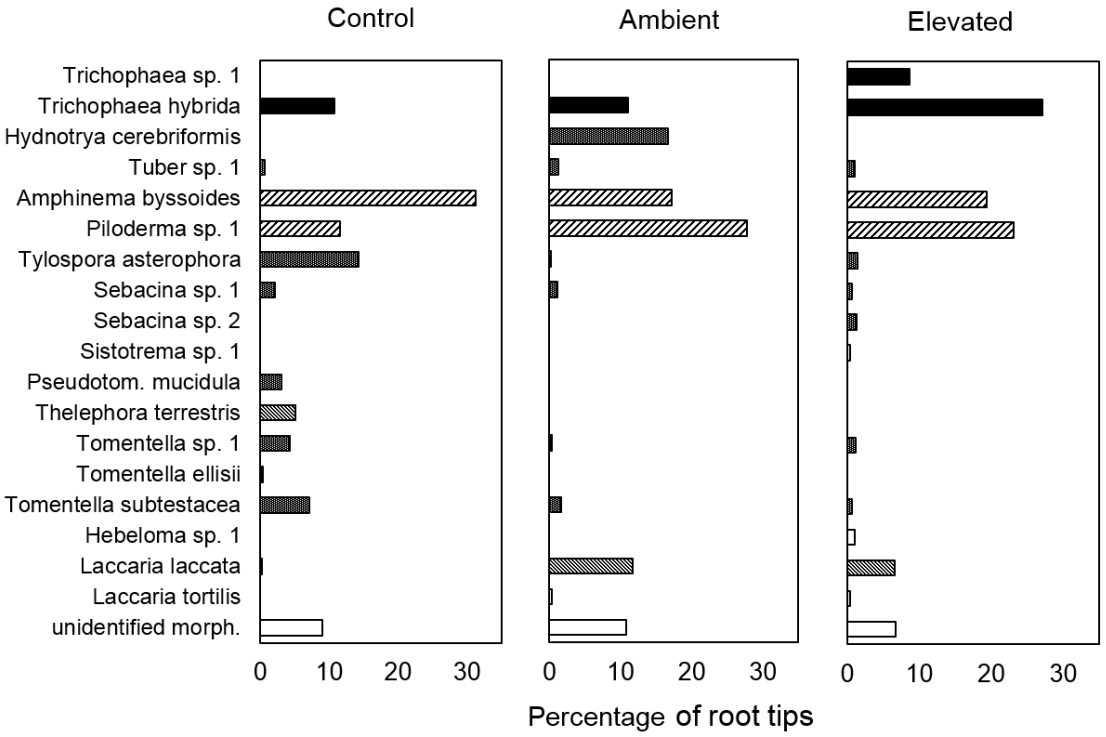

Figure 1. Ectomycorrhizal fungal species abundance as a percentage of total *P. abies* root tips colonised in glass domes in an unenclosed control, at ambient CO_2 or elevated CO_2 levels at the Bílý Kříž site. Exploration types: ■ contact, ▩ short distance, ▨ medium distance smooth, ▨ medium distance fridge, □ not known.

Canonical correspondence analysis (CCA) was carried out to determine which factors contributed to the differences in species occurrence and if these differences were statistically significant (Figure 2). The first axis of the CCA corresponds with the patterns of species occurrence between domes (Agaricales: *Laccaria*) and outside of the experimental area (Thelephorales: *Tomentella*, *Thelephora*, *Pseudotomentella*); the second axis shows the differences between the elevated CO_2 and ambient treatments. The occurrence of the species was significantly affected by site ($F = 2.33$; $p < 0.001$), but explains only 7.6% of all data variability (first axis 5.3%, second axis 2.3%).

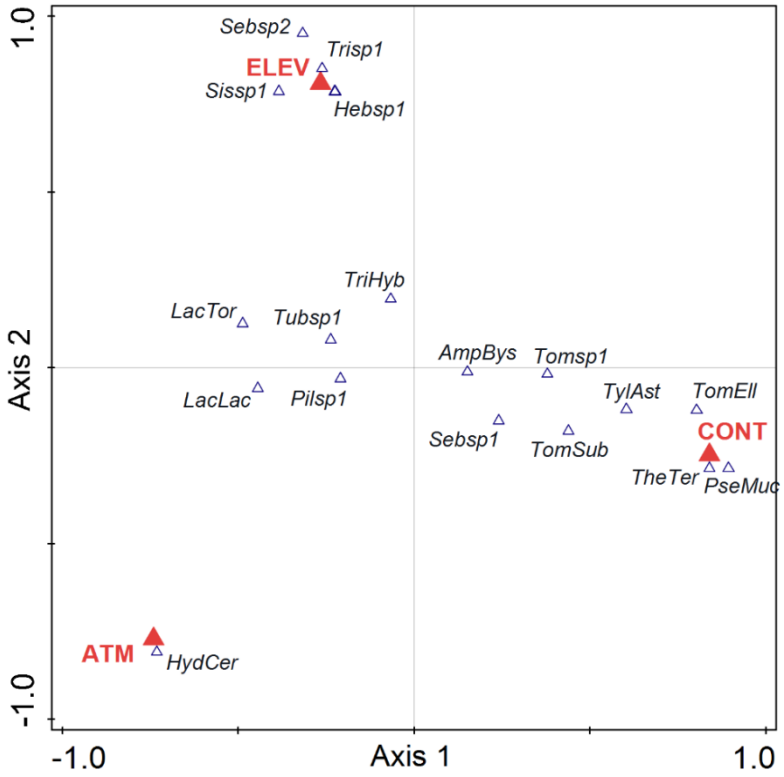

Figure 2. Canonical correspondence analysis diagrams showing the occurrence of morphotypes on the *P. abies* roots related to the site-glass domes at ambient CO_2 level (ATM) or elevated CO_2 level (ELEV) and in an unenclosed control (CONT) at the Bílý Kříž site. Species abbreviation: AmpBys, *Amphinema byssoides*; Hebsp1, *Hebeloma* sp. 1; Hydcer, *Hydnotrya cerebriformis*; LacLac, *Laccaria laccata* (Scop.) Cooke ; LacTor, *Laccaria tortilis* (Bolton) Cooke; Pilsp1, *Piloderma* sp. 1; PseMuc, *Pseudotomentella mucidula*; Sebsp1, *Sebacina* sp. 1; Sebsp2, *Sebacina* sp. 2; Sissp1, *Sistotrema* sp. 1; TheTer, *Thelephora terrestris*; TomSub, *Tomentella sublilacina* (Ellis & Holw.) Wakef.; Tomsp1, *Tomentella* sp. 1; TomEll, *Tomentella ellisii*; Trisp1, *Trichophaea* sp. 1; TriHyb, *Trichophaea hybrida*; Tubsp1, *Tuber* sp. 1; TylAst, *Tylospora asterophora*.

The percentage of root tips of contact exploration types increased from 12% in the ambient treatment to 35% in the elevated CO_2 treatment at the Bílý Kříž site (Figure 3). The increase in contact exploration types is mainly due to the increase in the occurrence of *Trichophaea* sp. 1 and *Trichophaea hybrida.* In the unenclosed control, there was a higher percentage of root tips of short exploration types compared to both the ambient and elevated CO_2 treatments. This was due to a higher abundance of *Tylospora asterophora*, *Thelephora terrestris* and three species of *Tomentella.*

Using redundancy analysis (Supplementary Materials, Figure S2), the differences in exploration type were shown to be statistically significant ($F = 3$; $p = 0.004$), even though, again, the factor treatment only explained 8.4% of the variation.

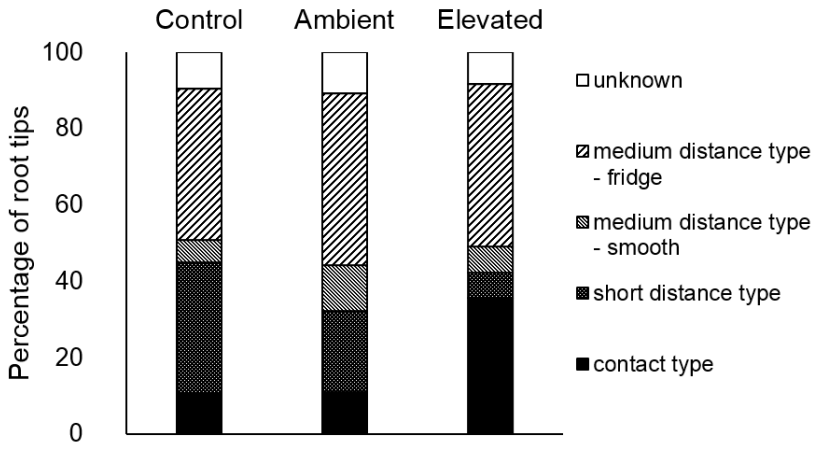

Figure 3. Ectomycorrhizal fungal exploration type abundance as a percentage of total *Picea abies* root tips colonised. The trees were grown in an unenclosed control or in glass domes at ambient CO_2 or elevated CO_2 levels at the Bílý Kříž site.

At the BangorFACE site (Figure 4), the roots of *Betula pendula* were dominated by the ectomycorrhizal fungi *Paxillus involutus* and *Inocybe* sp., which colonized 81%–84% of the root tips. No statistically-significant differences were found between the ambient and elevated CO_2 treatments, although the percentage colonisation for *Inocybe* sp. doubled under elevated CO_2. On the roots of *Fagus sylvatica*, five morphotypes were found; again, the dominant species was *Paxillus involutus* in combination with *Peziza sp*. A statistically-significant higher number of non-mycorrhizal root tips was found in the elevated CO_2 treatment compared to ambient conditions. Under elevated CO_2, the percentage of colonisation values of *Paxillus involutus* and *Peziza* sp. were not statistically significant for the ambient conditions.

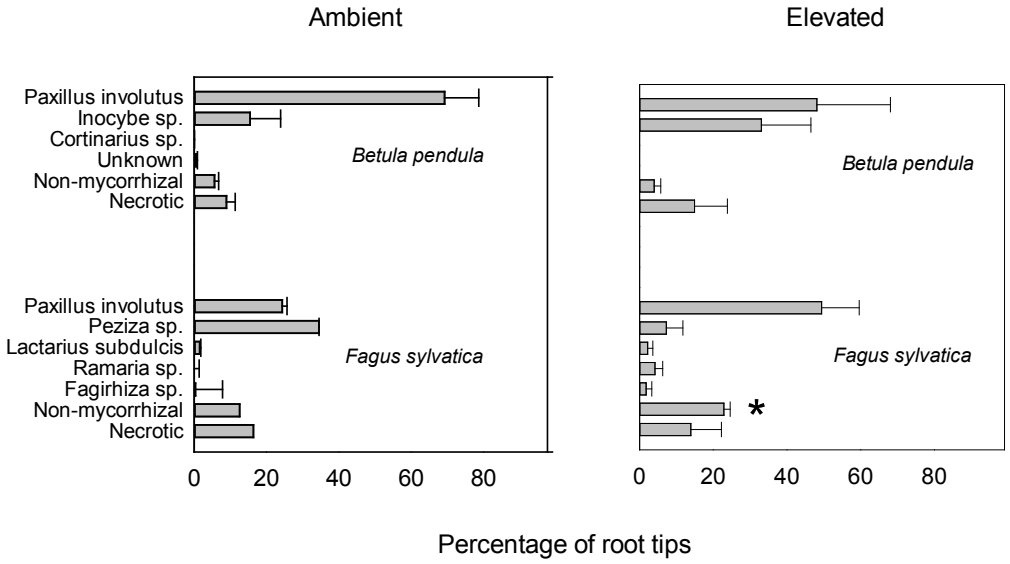

Figure 4. Percentage of colonisation of different mycorrhizal fungi on roots of *Betula pendula* and *Fagus sylvatica* at BangorFACE grown under ambient or elevated CO_2 levels (FACE). Bars show the mean and SE. * Statistically significant ($p \leq 0.05$).

Only in one year of the experiment were sporocarps formed, in 2006 (Table 1). Under elevated CO_2, the biomass of *Peziza badia* Pers. was significantly greater in elevated CO_2 compared to controls. The occurrence of sporocarps varied greatly between plots for *Peziza badia* and *Inocybe geophylla* (Sowerby) P. Kumm. In contrast, *Paxillus involutus* sporocarps were found in all plots of both the ambient and elevated CO_2 treatments. The total biomass of the *Paxillus involutus* sporocarps was 4.8-times higher under elevated CO_2, but due to the high variability between plots, this difference was not statistically significant. There was, however, a significant difference in the size of individual sporocarps ($p = 0.029$). In the ambient treatment, the mean sporocarp weight was 0.9 ± 0.1 g and in the elevated CO_2 treatment 1.9 ± 0.3 g.

Table 1. Total sporocarp biomass of fungi collected for ambient treatment and elevated CO_2 plots at BangorFACE in 2006.

Species	Sporocarp Biomass (g)		
	Ambient	**Elevated CO_2**	**E/A**
Paxillus involutus	29 ± 7	139 ± 61	4.8
Inocybe geophylla	0.04 ± 0.04	1.39 ± 1.05	32.0
Peziza badia	1.5 ± 0.6	8.9 ± 2.8 *	6.1

Shown are the mean ± SE. * Significant difference $p \leq 0.05$. E/A denotes the quotient of elevated over ambient.

4. Discussion

Between the two sites at BangorFACE and Bílý Kříž, there were differences in the species richness of the ectomycorrhizal species assemblage. A total of only eight morphotypes were found on the two tree species *Betula pendula* and *Fagus sylvatica* at BangorFACE, whereas a total of eighteen ectomycorrhizal species were identified on the roots of *Picea abies* at the Bílý Kříž site. Both *B. pendula* and *F. sylvatica* form ectomycorrhizal associations with a wide range of fungal species [17], and a richer ectomycorrhizal community was expected. The differences in species richness of the ectomycorrhizal fungal community between the two sites probably reflects a number of factors, including soil chemistry, such as soil P levels, but also inoculation potential of the soils and the surrounding area. In part, the higher species number at Bílý Kříž may also reflect the greater ease in determining rare species using molecular identification methods rather than morphotyping. BangorFACE was established on a former agricultural field with low ectomycorrhizal inoculation potential; the site at Bílý Kříž was established in a former *P. abies* forest with admixture of *F. sylvatica*. The soil used in the plots at Bílý Kříž was constructed from soil mineral layers and a litter layer from the surrounding forest with additional peat; this mixture has allowed a diverse ectomycorrhizal community to develop on young *P. abies* trees.

Irrespective of the identification method used or the complexity of the ectomycorrhizal community, elevated CO_2 induced changes in the community structure. At Bílý Kříž, there were changes in abundance in both the dominant and the rare species. Only a few studies have investigated the effects of elevated CO_2 on ectomycorrhizal community structure. In addition, the majority of these studies have been carried out in pot experiments that have a greater potential to introduce confounding artefacts [46]. In experiments where unrestricted root growth was permitted, change in the species assemblage was shown in *Betula papyrifera* and *Pinus strobus* saplings grown in a glasshouse [25]. In *Pinus taeda* L. grown under field conditions, *Tylospora* species decreased in abundance and *Russula* species increased

in abundance under elevated CO_2 [47]. After six years of CO_2 enrichment using FACE in *P. taeda*, Pritchard *et al.* [48] reported that ectomycorrhizal community structure had changed. However, this conclusion was not based on determination of fungal species, but rather that root tip morphology had changed. The change in root tip morphology was equated to a change in mycorrhizal fungal species.

An increase in fine root biomass [49] and an increase in the degree of mycorrhizal colonisation [25,46,50] is an often reported response to elevated CO_2. In contrast, ectomycorrhizal fine root tip colonization of *F. sylvatica* was significantly lower under elevated CO_2 than controls. In 2008, *F. sylvatica* had over 55% more root tips under elevated CO_2 than ambient conditions [35]. This suggests that the lower ectomycorrhizal colonization of root tips is a consequence of a greater stimulation of fine root growth than that of fungal growth. A higher number of non-mycorrhizal root tips was also found in *P. abies* grown under elevated CO_2 [9].

The ectomycorrhizal fungal community has been shown to change both in experiments with elevated CO_2 with a potentially higher C supply to the roots [9,25,51] and in defoliation experiments with a potentially lower C supply to the roots [52–54]. In both of these experimental manipulations, the change in ectomycorrhizal fungal community has often manifested itself as a shift between morphotypes differing in mantle thickness. A reduction in C flux to roots mediated through defoliation seems to favour smooth mycorrhizal types and disfavour types that produce thick mantles and rhizomorphs [52–54].

Under elevated CO_2, where an increased C allocation and flow below ground has been suggested [55], an increased proportion of mycorrhizas producing thick mantles and abundant rhizomorphs has been reported [25]. This response has never been shown in a field study; however, Pritchard *et al.* [56] showed that rhizomorph production was almost doubled by elevated CO_2 in deeper soil layers in a *P. taeda* forest. At Bílý Kříž, the increase in the occurrence of two *Trichophaea* species resulted in an increase in the percentage of contact exploration types in the ectomycorrhizal community. Contact exploration types have a low frequency or no presence of rhizomorphs; however, they are considered to have a higher biomass than short- or medium-distance ectomycorrhizal exploration types [57,58]. An increase in the frequency of contact exploration types is in agreement with the supposition that changes in ectomycorrhizal community composition under elevated CO_2 are due to an increased availability of C and an increase in the frequency of more C-demanding ectomycorrhizal species.

Surprising is the difference in ectomycorrhizal community composition between the domes and outside control area (Figures 1 and 2). Two factors may have influenced ectomycorrhizal community composition: minor microclimate and soil property differences (less snow and slightly higher maximum air temperature and higher soil humidity within domes) and spore accessibility. Since there is very limited knowledge about the autoecology of ectomycorrhizal fungal species (most of them do not form easily visible sporocarps), it is difficult to interpret the observed differences. Several species, such as *Piloderma* sp. 1, *Amphinema byssoides*, *Tylospora asterophora* and *Tomentella ellisii*, were also found as mycorrhizas in the surrounding forests. *Laccaria laccata* was found as a sporocarp in the surrounding forest [59]. *Amphinema byssoides*, *Tuber* sp. and *Thelephora terrestris* are common mycorrhizas of nurseries [60]. The original ectomycorrhizal colonization of the *P. abies* seedlings planted in the domes and control areas came from the nursery. It is possible that over the course of time, the evenly colonized seedlings from the nursery were exposed to different spore flow levels in the three treatment types, which has influenced mainly the species composition, whereas microhabitat conditions (CO_2 level, temperature, humidity) have influenced species abundance. The difference in ectomycorrhizal

community structure between the domed treatments and the non-domed control indicate that, in agreement with the work of Andrew and Lilleskov [61], small differences in microclimatic conditions can have comparable effects on the composition of the ectomycorrhizal community as that of an increased level of CO_2.

According to Andrew and Lilleskov [62], sporocarps can be significant carbon sinks for ectomycorrhizal fungi and are last in the plant C flux continuum that starts with C fixation by photosynthesis and ends with exudation or respiration. Andrew and Lilliskov [62] could show that in *Populus tremuloides* Michx. and *B. papyrifera* stands, total sporocarp biomass increased under elevated CO_2. The increase in total sporocarp biomass was due to an increase in the biomass of later successional species (e.g., *Leccinum* sp. and *Cortinarius* sp.), whereas the biomass of early successional species (e.g., *Hebeloma* sp., *Paxillus involutus* and *Inocybe lacera* (Fr.) P. Kumm.) decreased. At BangorFACE, the root tip ectomycorrhizal community was, with the exception of *Cortinarius* sp. and *Lactarius subdulcis* (Pers.) Gray, composed of early successional mycorrhizal fungi [63]. Total sporocarp biomass also increased under elevated CO_2, and significant differences were found for the biomass of *Inocybe geophylla* and the average individual sporocarp biomass of *Paxillus involutus*. The three species that produced sporocarps, *Paxillus involutus*, *Inocybe geophylla* and *Peziza badia*, dominated the belowground community, but did not significantly increase in abundance under elevated CO_2. Clearly, there was a greater C flow belowground, as evidenced by the increase in production of sporocarp biomass, and there were a sufficient number of root tips available for colonization, as shown by the number of non-mycorrhizal root tips in both *B. pendula* and *F. sylvatica*. A possible reason for the observed lack of change in abundance of mycorrhizal species is competition for, or partitioning of, available C between mycorrhizal biomass components (e.g., mantle, hyphae, rhizomorphs and sporocarps).

This work shows that at two different sites planted with different tree species, elevated CO_2 led to changes in the ectomycorrhizal community structure, shown most clearly at Bílý Kříž, and an increase in sporocarp biomass, as shown at BangorFACE. Both of these effects may be mediated by a greater C flow belowground. There is a suggestion that even with this greater belowground C allocation, there is still competition for C between root growth, root tip mycorrhizas and sporocarp production.

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Author Contributions

Douglas Godbold instigated the BangorFACE experiment, analysed the data and led the writing of the paper. Martina Vašutová collected the root samples at the Bílý Kříž site, identified ectomycorrhizal morphotypes, did the molecular analysis and participated in the writing. Anna Wilkinson collected the root samples and identified ectomycorrhizal morphotypes at BangorFACE. Magda Edwards did statistical processing of the data from the Bílý Kříž site. Pavel Cudlin coordinated root and mycorrhizal research at the Bílý Kříž site and participated in the writing. Marian Pavelka processed environmental data from the Bílý Kříž site. Michael Bambrick collected and identified the sporocarps at BangorFACE. Andrew Smith led the work carried out at the BangorFACE site.

Conflicts of Interest

The authors declare no conflict of interest.

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